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L1-CAM is commonly expressed in testicular germ cell tumors

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Abstract

Germ cell tumors (GCT) are curable cancers, but 10-15% of patients with metastatic disease fail cisplatin-based first-line therapy. While therapeutic options have increased for various other cancers no therapeutic targets have emerged in cisplatin-refractory GCT. L1 cell adhesion molecule (L1-CAM) is commonly expressed in human malignancies and therefore a potential target, however its expression in GCT has not been studied so far. The aim of our study was to describe the expression of L1-CAM in a large series of testicular GCT. Immunohistochemistry was used to study L1-CAM expression in 325 testicular GCT, including 94 mixed GCT. L1-CAM expression was found in 38% of seminomas, 50% of yolk sac tumors, 19% of teratomas, 50% of choriocarcinomas, 67% of embryonal carcinoma. L1-CAM was expressed in 45% of germ cell neoplasias in situ but not in normal tissue. This common L1-CAM expression in testicular GCT might serve as an immunotherapeutic target in the future.

INTRODUCTION

In 2015, an estimated number of 8,430 new cases of GCT will be diagnosed in the United States [1]. Although GCTs show a high sensitivity to cisplatin-based chemotherapy, 10-15% of patients fail first-line chemotherapy and 3-5% of all GCT patients will eventually die of their disease [2]. Despite a response rate above 95% to cisplatin based chemotherapy, the search for new treatment strategies remains worthwhile in accordance to reduce treatment toxicity and offer therapeutic options in non-responding patients [3-5]. Various tumors have been described to express L1-CAM including lung carcinoma, gliomas, melanoma, renal, ovarian, endometrial and colon carcinoma [6]. L1-CAM is associated with tumor cell dissemination via the regulation of pro-metastatic MMP-2 and MMP-9 in solid and non-solid tumors [7] as well as in brain metastases[8]. L1-CAM is involved in epithelial to mesenchymal transition (EMT)[9]. In various malignancies there is evidence showing that expression of L1-CAM is associated with a subset of highly aggressive tumors with adverse clinical outcome and might serve as a therapeutic target[10]. We aimed to investigate the expression of L1-CAM in the different GCT subtypes.

MATERIALS AND METHODS

The construction of the tissue micro array (TMA) was described before [9]. L1-CAM immunohistochemistry (IHC) was performed using the monoclonal antibody anti-L1-CAM (clone 14.10, directed to the ectodomain, 1:200). The antibody was generated as described previously [11] and was tested on a multi tissue TMA for the appropriate dilution. Peripheral neuronal tissue served as internal positive control for L1-CAM staining. Sertoli cells and Leydig cells were negative for L1-Cam. Two experienced surgical pathologists (PKB, VT) evaluated the L1-CAM stained TMA. Samples were dichotomized into positive versus negative. The threshold for positivity was defined at 5% of cells with a moderate or strong staining. The expression patterns were separately analyzed for each tumor component, GCNIS and normal tissue. The study was approved by the local ethics committee (reference number KEK StV. 25-2008).

RESULTS

The series included a total of 207 seminomas, 4 spermatocytic tumours, 19 pure embryonal carcinomas, 1 pure mature teratoma and 94 mixed GCT. Mixed germ cell tumors included the following components: seminoma, embryonal carcinoma, yolk sac tumor, choriocarcinoma and teratoma (49 with and 45 without a seminomatous component).

The individual tumor components consisted of 253 seminomas, 89 embryonal carcinomas, 52 yolk sac tumors, 53 teratomas, 10 choriocarcinomas. In addition, we included non-tumorous testicular tissue from 20 tumor patients and GCNIS from 20 patients.

L1-CAM IHC staining showed an exclusively membranous staining pattern in GCTs with moderate to strong intensity. In seminoma, IHC staining of L1-CAM showed a predominantly heterogeneous membranous pattern and 95 cases (38%) were L1-CAM positive (see table and figure 1).

Tissue types	Negative	Positive	Positive cases in %
Normal testis (n=20)	20	0	0%
Intratubular germ cell neoplasia (n=20)	11	9	45%

Seminoma (n=253)	158	95	38%
Choriocarcinoma (n=10)	5	5	50%
Yolk sack tumor (n=52)	26	26	50%
Embryonal carcinoma (n=89)	29	60	67%
Teratoma (n=53)	43	10	19%

Table 1 L1-CAM expression in non-neoplastic and neoplastic testicular tissue

The yolk sac tumors with microcystic, glandular, solid and spindle cell growth patterns showed a heterogeneous expression of L1-CAM in 26 cases (50%). The glandular components were positive in a L1-CAM staining whereas areas with stromal or spindle cell differentiation were negative. The results of L1-CAM staining in teratoma components were very heterogeneous depending on the tissue types found in the teratoma. Ten cases (19%) showed L1-CAM positive structures, e.g. glands with a membranous pattern. Stromal components as well as chondrocytes were L1-CAM negative. Five (50%) choriocarcinoma cases expressed L1-CAM. The expression was more restricted to the syncytiotrophoblastic giant cells whereas the mononuclear component was predominantly negative. Interestingly, syncytiotrophoblastic giant cells were also positive when scattered in a seminomatous tissue component. In embryonal carcinoma 59 cases (63%) were positive with an intense and homogenous pattern. Normal testicular tissue was L1-CAM negative whereas IGCNU showed a strong expression of L1-CAM in 45% of all cases.

DISCUSSION

Recently, L1-CAM has emerged as a potential therapeutic target due to its expression on many solid tumors, and only limited expression on normal tissues[6]. *In-vitro* and *in-vivo* studies showed efficacy and safety of L1-CAM targeting antibodies by acting via antibody-dependent cellular cytotoxicity (ADCC) or by being labeled with radionuclides[12-23]. In a humanized transgenic mouse model of L1-CAM no adverse effects were observed after injection of ant-L1-CAM antibodies [24]. A first in human phase I trial was

published by Park et al showed no adverse effects and some objective response after infusing autologous CE7R/HyTK+ CD8+ cytolytic T-lymphocytes [20]. However, it cannot be concluded from this study, if the response was due to the L1-CAM therapy or the subsequent salvage therapies.

We observed that L1-CAM expression is markedly enhanced in most GCTs, in 45% of GCNIS but not in normal tissue. As typical for a retrospective study, this investigation is limited by potential biases, such as patient selection and bias of core punching.

To our knowledge this is the first publication describing L1-CAM expression in GCTs. We conclude that the frequent expression of L1-CAM in testicular seminomas and non-seminomas indicates that L1-CAM could be a promising new therapeutic target in testicular cancer that warrants further functional studies and potentially investigation in clinical trials in the future. Because L1-CAM was never expressed on normal but frequently on cancer cells, further investigation should elaborate the role for L1-CAM as a new "neoplastic" germ cell tumor marker.

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Figure Labelling

Figure 1. L1 cell adhesion molecule (L1-CAM)staining in several tumor components: Classic seminoma with heterogeneous membranous (A); Embryonal carcinoma with typical homogenous and intense membranous staining pattern (B); Yolk sac tumor with weak heterogeneous membranous staining (C); choriocarcinoma with strong membrane staining in dispersed tumor cells, predominantly multinucleated giant cells (D); teratoma with heterogeneous membranous staining (E); strong staining in the basal part of the tubuli in carcinoma-in-situ (CIS) germ cells (F)

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